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Research Article

Non-Alcoholic Extraction of Rose Pigment as a Halal and Safe Natural Colorant and Bioactive Compound

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ABSTRACT

Rose is a well-known cut ornamental flower. The rose crown has been known to contain high anthocyanin, particularly cyanidine and dephinidine-glycoside. Those bioactive compounds have been proven to act as free-radical scavengers. Low grade and non-fresh flowers still contains potential pigments, which can be utilized as natural colorant for food and supplement such as effervescent tablet. The study was to investigate the effect of post-cutting process on the quality of anthocyanin extract and the quality products, and to examine bioactivity of rose effervescent tablets on the liver protection through SGPT test using white rat. The study consists of three experiments. First experiment was aimed to investigate the effect of pigment quality derived from rose crown, *i.e.* fresh cut rose, 2 and 4 days after cutting. Further, the pigment was then applied on several products (*i.e.* fruit juice, jelly and carbonated drink or effervescent tablet) and the qualities were examined in second experiment. Lastly, the third experiment deals with pre-clinical examination using white rats. The study showed that anthocyanin pigment of rose in the dosage of 3% significantly contributed to formation of a red and yellow color in those industrial products. Effervescent tablet derived from local rose significantly decreased SGPT level of white rat wistar strain induced by CCl_4.

1. INTRODUCTION

Food coloring can be either natural or artificial. The artificial color may have originated synthetic compounds that were added into the food, while natural colorant is originated from the compounds that naturally present in the food materials. While the synthetic one is human-made compounds, the natural colorant is considerably granted by Allah Almighty to the nature. Some messages of the God in the Al-Qur'an also remind all the human to explore the nature, as written on Surah Ar-Ra'd (13) verse 4: "and in the earth are tracts (diverse though) neighboring, and gardens of vines and fields sown with corn, and palm trees-growing out of single roots or otherwise: watered with the same water, yet some of them We make more excellent than others to eat. Behold, verily in these things there are signs for those who understand"; also on Surah An-Nahl (26) verse 69: "Then eat from all the fruits and follow the ways of your Lord laid down [for you]. There emerges from their bellies a drink, varying in colors, in which there is healing for people. Indeed, these are a sign for us to think" (this translation Saheeh Al-Quran). Beverages with variety of color are supposed to contain pigments.

According to the National Law of Food Regulation in Indonesia No.7/1996, food safety is defined as a condition and an effort needed to prevent food product from any possibility of biological, chemical, and other contaminations that may be harmfulto human. Food safety is related to food sanitation, food additive, genetic engineering, food irradiation, food packaging, quality assurance, lab controlling, and contaminated food (Ministry of Health of Indonesia, 2000). In fact, violation of the law of food regulation by some food industries in Indonesia, especially for the use of artificial colorants is the second biggest issue of food safety (11.31%), after the use of artifical sweeteners (21.45%) (BPOM, 2006). Moreover, non-food grade colorants, such as rhodamin B, methanil yellow, and amaranth, are still freely distributed in food market. They have been actually banned since they may cause serious risks for human health.

Anthocyanin is a potential water soluble pigment widely distributed in many natural products. It is more stable in acidic pH ranging from 1 to 4, in which it reflects not only a red color, but also orange, pink, purple, and blue (Li, 2009; Lewis *et al*., 1997). Rose (*Rosa sp.*) is one of cut-flowers mostly used as ornamental flowers for formal events, such as seminar and workshop; as well as non-formal events such as wedding party and some traditional ceremonies. The crown of local roses originated from Batu has been known containing anthocyanin in particular for the type of cyanidine and dephinidine-glycoside (Saati *et al.,* 2007) as well

as malvidine-glycoside (Saati, 2011). It is also used as natural medicine (Rukmana, 1995). Blake (2004) and Tanaa *et al.* (2009) also stated that rose contains high amount of cyanidine (cyanines). Anthocyanin containing in rose is expected as a potential natural colorant that is healthy, safe, and halal for human. During 2006-2013, Saati and co-workers have been successfully extracted pigment from rose flower without alcoholic solvents to provide a halal colorant product. In developed countries, the utilization of natural colorant has been applied intensively in various products such as foods, medicines, supplements, cosmetics, feeds and handicrafts (US Patent No. 20090246343, Wu *et al.*, 2008).

Nowadays, our environment has been contaminated by high concentration of free radicals. Rachmawati (2003) stated that increasing free radical compounds production may cause dysfunction on some internal organs such as liver and kidney. In order to inhibit free radical formation, it is necessary to use antioxidant agents as protector of organs damage due to free radical compounds. Pigment as a bioactive compound is not only useful for human heath, but also as natural colorant that can be further developed as supplement such as effervescent tablet. Since anthocyanin is highly water soluble, it will be easily absorbed by the human body, and thus anthocyanin is categorized as one of the natural products that is mostly used in human diets. Furthermore, several studies also revealed that anthocyanin could prevent heart disease, cancer, hyperlipidemia and other chronic diseases (Downey *et al.,* 2006; Soni & Maria, 2007), such as diabetes and stroke (Garz'on *et al.,* 2009). Therefore, this research is useful for exploring further utilization of anthocyanin derived from local rose as natural colorant and bioactive product (antioxidant), thus it may promote local natural resources to provide healthy, safe and halal food product as well as increase local income.

The aims of this study were to investigate the effect of post-cutting process on the quality of anthocyanin extract of local roses, to investigate the effect of rose anthocyanin on the quality of three different products, and to examine bioactivity of extract of local roses and rose effervescent tablets on the liver protection through SGPT test using white rat.

2. MATERIALS AND METHODS

The study was conducted in Laboratory of Food Science and Technology and Laboratory of Chemistry, University of Muhammadiyah Malang; Laboratory of Chemistry, Polytechnic of Malang; and Laboratory of Pharmacology University of Brawijaya, Malang. Fresh local red roses from Punten Village, Junrejo District, Batu, Indonesia was used as main material. Distilled water, citric acid, petroleum ether, ethanol, HCl, BAA, and BuOH-HCl as mobile phase were used as solvents for pigment extraction, isolation, and analysis. DPPH (2-2-diphenyl-2-picryl hidrazil). Maltodextrin, sodium-bicarbonate, and sucrose were used to make effervescent tablet. The equipment used were glassware, digital balancing, centrifuge, vacuum filter VWR scientific, vacuum rotary evaporator, spectrophotometer UV VIS Shimadzu, pH meter CG 832 Scholl Gerale, Color Reader CR-10 Konica Minolta (provided in Laboratory of Food Science and Technology, University of Muhammadiyah Malang), and Thin Layer Chromatography (provided in Laboratory of State Polytechnic of Malang)

The study consists of three experiments. First experiment was non-alcoholic extraction of pigments and analysis of pigment quality derived from different condition of rose crown, *i.e.* fresh cut rose (U_0), 2 days after cutting (U_1), and 4 days after cutting (U_2). Complete randomized design was used as experimental design in this work.

Second experiment was evaluation of the extracted-pigment application on several products, including fruit juice, jelly and supplement in the form of effervescent tablet. For this experiment, randomized block design was used as experimental design consisting of2 factors, *i.e.* pigment concentration (A): 0, 2, 4%, and type of product (B): fruit juice, jelly and carbonated drink, then to examine bioactive test for effervescent tablet. All experiments were carried out with three replications.

Pigment was extracted from rose crown using mixed solvent of distilled water and citric acid with the volume ratio of 95:5. It was then stored at temperature of 10~12°C for 30 minutes in order to let pigment extracted optimally. The mixture was filtered using cheese cloth and pressed. The filtrate was further filtered using filter paper Whatman no. 41 in a vacuum condition. The clarified filtrate was added 0.2~1% of petroleum ether for separating non-anthocyanin compounds. Dried pigment powder was obtained by mixing the pigment extract and dextrin 30% as a filler. Some parameters of quality analysis were pH, moisture content (AOAC, 1995; Sudarmadji, 1998), solubility (AOAC, 1995; Sudarmadji, 1998), absorbance (Jenni *et al.,*1997), color intensity (Fabre *et al.,* 1993), pigment yield (Markakis, 1982) and bioactivity or antioxidant power with radical scavenging activity (RSA) test (Konga *et al*., 2007).

The last experiment or the final step was pre-clinical examination used white rats (*Rattus norvegicus*) (wistar strain) that had been acclimitized for a week and divided into 5 groups labelled as control, CCl_4, dosage I, dosage II and dosage III. Each tablet of effervescent was 5g in weight containing 4.85mg of anthocyanin that was packed in aluminium foil. The dosages of effervescent tabet used were 1.25g, 2.5g and 5g containing 1.21g, 12.12g and 24.25mg of anthocyanian, respectively. Effervescent tablet containing red rose pigment was adminstered to the rats by oral, while CCl_4 in the dosage of 0.195 ml/150gBW/3 days was administered for 14 days by injection. The value SGPT (serum glutamic pyruvic transaminase) was analyzed prior and post tests.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of Local Red Rose

The fragrant coming out from the crown of rose is due to its several of essential oils, but still containinganthocyaninwhich binding sugar. Previous study demonstrated that essential oils from hybrid and local rose were about 0.8%. Blake (2004) also stated that rose containing total sugar (8~12%) which was bonded to cyanins and essential oils (in a range of 0.06~1.0%) was composed of citronellol, eugenol, galic acid and linalool. The average of total sugar, moisture content and essential oils of the rose in this study was presented in Table 1.

Table 1. The average of total sugar, moisture content and essential oils in rose crown

Treatments	Total sugar (%)	Moisture content (%)	Essential oil (%)
U_0 (Fresh cutting)	8.33± 0,62a	85.08±0,96	0.80±0,64b
U_1 (2 days post cutting)	8.87± 0,63a	84.62±0,98	0.78±0,63b
U_2 (4 days post cutting)	10.97± 0,66b	79.58±0,99	0.44±0,61a

Note: The same letter on the same column indicated insignificant result according to Duncan Multiple Range Test (DMRT) 5%.

During storage period for 4 days (room temperature), moisture content of crown rose decreased about 15% (5 gr/300 ml, m/v) and total sugar increased more than 2%. The reduction of moisture content was due to the transpiration process as the mechanism of catabolism to gain adequate energy to survive. The transpiration process also involves withering stage in which some essential oils may evaporate. On the other hand, this process is able to increase sugar content as indication of presence of anthocyanin which is bound sugar molecule as glycone group. De Man (1997) stated that anthocyanin pigment could be hydrolyzed into aglycon and glycone forms that they belonged to sugar molecules.

3.2. Analysis of Quality of Rose Pigment (Concentrated and powdered pigments)

3.2.1. Absorbance of pigment

The results showed that post cutting process significantly affected to the absorbance of both concentrated and powdered pigments. The average of pigment absorbance of the treated rose was presented in Table 2.

Table 2. The average of absorbance of red rose pigment on the post cutting treatment (λ = 525 nm)

Treatments	Absorbance	
	Powdered pigment	Concentrated pigment
U_0 (Fresh cutting)	1.090a	0.423a
U_1 (2 days post cutting)	1.180b	0.487b
U_2 (4 days post cutting)	1.220b	0.540c

Note: The same letter on the same column indicated insignificant result according to Duncan Multiple Range Test (DMRT) 5%.

Table 2 showed that pigment of rose originated from 4 days post cutting exhibited the highest absorbance, namely 1.220 and 0.540 (100 times dilution) for the powdered and concentrated pigments, respectively. These data were also corelated to the moisture content. The lowest moisture content (4.57%) was shown from 4 days post cutting samples compared to those of the fresh cutttingones (4.68%). The absorbance of the pigment powder increased as the longer storage period after harvesting. The increase of the absorbance was due to reduction of water content as well as an indication that the higher content of anthocyanin in pigment powder. Budiarto (1991) revealed that the changing of pigment absorbance depended on soluble anthocyanin concentration and formation changing of anthocyanin structure. If pigment appeared red color, the anthocyanin might be still stable. Otherwise, when it turned into colorless, the anthocyanin has been degraded. Type and amount of anthocyanin containing in the plant tissue depends on species, variety, maturity, location, etc. (Tranggono, 1990).

3.2.2. Intensity of color of rose's pigment

The results showed that post cutting process significantly affected to the color intensity of both concentrated and powdered pigments, including brightness level (L) and redness level (a+). The average of color intensity of the treated rose was presented in Table 3.

Table 3. The average of brigtness (L) and redness (a+) of red rose pigment on the post cutting treatment

Treatments	Color intensity of powdered pigment		Color intensity of concentrated pigment	
	L	a+	L	a+
U_0 (Fresh cutting)	62.23b	26.53a	39.20 c	33.17 a
U_1 (2 days post cutting)	61.43a	29.63b	37.67 b	34.17 b
U_2 (4 days post cutting)	60.73a	32.83c	35.90 a	35.27 c

Note: The same letter on the same column indicated insignificant result according to Duncan Multiple Range Test (DMRT) 5%.

According to some parameters observed during study, the pigment extracted from 4 days post cutting sample performed better quality than that of fresh cutting one, either processed into powder or concentrate product. The absorbance of its powder pigment, redness, total soluble solid and low moisture content was 1.22, 32.82, 9.07% and 4.57%, respectively. Meanwhile, its concentrated pigment also indicated high quality in redness and brightness, with 35.27 and 35.9 score levels, respectively. This study proved that low grade cut rose, that was previously considered as low economic materials, even as a waste in cut flower home industry, in fact it was contained a much higher anthocyanin content compared to that of the high grade cut rose (the fresh one). Extension of storage time after cutting process might cause evaporation of some essential oils and thus anthocyanin content increased. Essential oils and anthocyanin possessed different properties. Essential oil is water insoluble that can be dissolved in organic solvent (Fennema, 2000), while anthocyanin is water soluble (Henry & Houngton, 1996).

3.2.3. Application of pigment in industrial products

Anthocyanin pigment from red canna flower is stable contributing red and orange color in pH range of 1~11 (Saati, 2007), even though Shi *et al.* (1992) stated that anthocyanin is more stable in acidic pH in range of 1 to 5. Therefore, in this study rose anthocyanin was applied in some industrial product in pH range of 1~11. The results showed that some selected industrial products have had pH level in range of stable pH of anthocyanin (Saati *et al*., 2007) and have contributed its natural color of red and yellow. Table 4 showed the application of anthocyanin pigment extracted from local red rose contributed in product color by 2~3%.

Table 4 also presented quantitative data of the effect of anthocyanin application in three different products on color appearance of the products. The results showed a positive response on the product appearance due to anthocyanin fortification, which was indicated by less brightness (L) and more redness as the main character of anthocyanin pigment. Lewis *et al.* (1997)

defined that anthocyanin is a pigment of red, orange, purple and blue. It was also supported with a patent by Wu *et al.* (2008) who stated that natural colorant could be used in many products applications for instances: food, beverages, medicine, including supplement such as effervescent tablet.

Table 4. Color intensity of some selected industrial products applied without and with 3% of anthocyanin pigment

Products/treatments	L value	Redness	Yellowness
A0B1:Fruit juice without anthocyanin	29.50	4.40	8.80
A1B1: Fruit juice with 3% of anthocyanin	28.47	5.13	9.00
A0B3: Jelly without anthocyanin	24.47	4.00	7.07
A1B3: Jelly with 3% of anthocyanin	23.30	3.30	7.43
A0 B4: Carbonated drink without anthocyanin	34.90	-1.30	2.60
A1B4: Carbonated drink with 3% of anthocyanin	29.30	4.90	3.10

3.2.4. RSA test of extract of local roses

RSA (radical scavenging activity) test is one of analysis for antioxidant power by added DPPH (2-2-diphenyl-2-picryl hidrazil) to sample (Konga *et al*., 2007).The observations results of antioxidative power to extract pigments, pigment powder until tablet effervescent of red rose crown ascribed from local varieties of Batu city, are shown in Table 5.

Table 5. The average of bioactivity/antioxidant power of red rose pigment to pigment tablets (λ = 525 nm)

Treatments	Antioxidant power (%)	
	Antioxidant power	Decrease of antioxidant power
Concentrated pigment (Aquades % citrate acid)	79.07	start point (0)
Powdered pigment	28.60	63.83
Tablet effervescent	17.20	78.25

Bioactivity test showed that the antioxidant power of red roses pigment extract has the highest value of 79.07%, while the longer stages of processing it will decrease the power of antioxidants, such as into a powder pigment fell 63.83% to 28.6(%) and continues to drop again (78.25%) after becoming a tablet efferevescent be only 17.2(%) antioxidant power. It is the nature of anthocyanins contained in red roses. Anthocyan instability can be affected by several factors such as: the structure and concentration of anthocyanins, acidity (pH), oxidizing agents, light, temperature and so on (Jackman and Smith, 1996).

3.2.5. SGPT test of effervescent tablet of local rose on white rats

Pigment as bioactive compound is useful for medicinal purpose or supplement to improve human health (Mohre, 1990). A study by Raminez *et al.* (2001) showed that anthocyanin has antioxidant activity 3~4.5 higher than vitamin E. Therefore, apart from its function as natural colorant, we expected that anthocyanin contained in effervescent tablet of local rose can play a role as bioactive product that promotes a human health through its antioxidant activity.

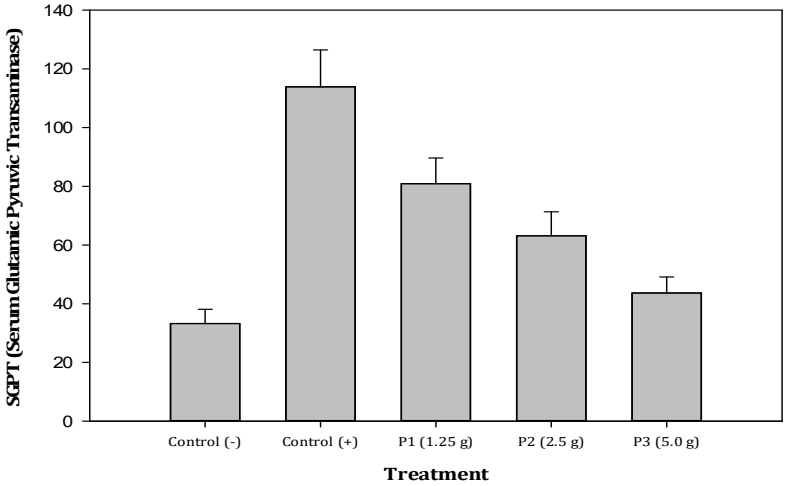

Figure 1. The effect of rose effervescent tablet on SGPT level of rats

SGPT (serum gutamic Pyruvic Transaminase) is an enzyme present in many cells, but majority in cytoplasm of liver cells. Results of SGPT test are presented in Figure 1. According to the One Way ANOVA test in this study, there was no significant difference ($p<0.05$) in SGPT level. Meanwhile, according to Post Hoc analysis, there was a significant difference between Control (-)(without CCl_4) and Control (+)(with CCl_4). Nevertheless, there was no significant difference between final percent of Control (-) and P3 (Rats treated with CCl_4 with the dosage of 0.195ml/150gBW/3 days, followed by administration of rose effervescent tablet with the dosage of 5g). The significance value was 0.323, which means that SGPT level between control group and P3 group were not significant different. It revealed that the dosage of rose effervescent tablet by 5g was effective enough in reducing SGPT level up to close to normal level.

4. CONCLUSION

Concentrated and powdered pigment of anthocyanin extracted from rose crown after 4 days cutting displayed better quality than that of the fresh cut one. It was indicated by the absorbance of 1.22, redness of 32.83 and moisture content of 4.57%. Anthocyanin pigment of rose in the dosage of 3% was able to contribute a red and yellow color in some industrial products such as fruit juice, jelly and carbonated drink or effervescent tablet.

The antioxidant power of red roses pigment extracthas the highest value while the longer stages of processing it will decrease the power of anti oxidants andcontinues to drop again afterbecoming a tablet effervescent. Effervescent tablet derived from local rose significantly decreased SGPT level of white rat wistar strain induced by CCl_4.

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